Summary

The project aimed to assess the potential of inoculating seed with a soil organism to improve the ability of crops to access phosphorus (P) from the soil. The research improved understanding of the organisms used and their impact on P availability and crop growth. Results indicate that the organisms used in this work can improve the early growth of cereal crops on acidic to neutral soils but have little benefit in P management.

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Conclusions

- Inoculation of wheat (and other crop) seed with the fungal inoculant Penicillium radicum can significantly increase plant growth and yield (increasing returns to growers).

- Inoculation with P. radicum is most successful on neutral to acidic soils with a low disease background. Strongest effects are seen on acidic soils that may be considered as being of ‘low fertility’.

- P. radicum is ineffective on calcareous and alkaline soils.

- P. radicum does not exhibit biological control efficacy. In fact, in the presence of soil borne plant pathogens the plant growth promoting (PGP) activity of this fungus is removed.

- P. radicum is relatively ineffective at solubilising phosphate minerals. In numerous glasshouse studies, PGP activity was not associated with increases in foliar phosphorus (P) concentrations or total P uptake. Along with evidence supplied by Simon Anstis (ARC-SPiRiT funded PhD project at CSIRO and Adelaide Uni), it is now apparent that PGP by P. radicum is not associated with P-solubilisation. More likely, P. radicum stimulates the branching of fine roots, allowing inoculated plants to explore more of the soil and thereby capturing available nutrients (but not by making nutrients more available per se).

- Growers should not use P. radicum with the presumption of gaining higher P-fertiliser efficiency or accessing P locked up in soils.

- New strains of Penicillium have been discovered with much higher phosphate solubilising activity than P. radicum. These isolates increase the growth and P nutrition of a variety of crop plants (including wheat, medic and lentil) across a range of soil types. These new isolates of Penicillium increase plant growth on alkaline soils on which P. radicum is not recommended for use.

- New strains of Penicillium have PGP efficacy in the field (yield increase of 10% on lentil in 2003), are easy to culture/formulate and are, therefore, valuable to industry.

- The potential of the new strains of Penicillium to be of value to the Australian grains industry is evident from overseas success. A strain of one of the new phosphate solubilising fungi isolated in this project is already widely used as a phosphate solubilising inoculant in North America.

Recommendations

- Growers use Penicillium radicum to increase early plant growth on acidic to neutral soils where soil borne disease management strategies are in place. Vigorous early growth should allow plants to better capture soil nutrient resources (nutrients and water), compete with weeds and resist diseases, often resulting in increased yield.
- Growers should not use Penicillium radicum to manage P in cropping systems.
- New strains of Penicillium are much better suited for the role of P management than Penicillium radicum. One of these strains has been developed overseas (Canada). These new strains should be considered for development as phosphate solubilising inoculants in place of P. radicum, which should be marketed and promoted as a general plant growth promoting fungus.
- The new Penicillium inoculants significantly increase the growth and yield of legumes (numerous glasshouse trials, field trial 2003). As such, the feasibility mixing these inoculants into current rhizobium products should be explored, particularly as this has been shown to be highly successful overseas. Products containing Rhizobia and Penicillium have many advantages: they reduce cost by removing the need for dual inoculation; growers are already familiar with the use of peat-based inoculants; it provides a vehicle for growers to ‘test’ Penicillium inoculants with little extra effort or risk; there are considerable synergistic benefits from having N fixing and P solubilising activities occurring together in the plant rhizosphere; it adds value to existing rhizobia inoculant products. Once established and accepted, Penicillium (only) inoculants may be more easily ‘bridged’ into the wider grain market.
- To enable the successful commercialisation and grower adoption of the new Penicillium inoculants, further research is needed.

**Outcomes**

**Economic outcomes**

1. Key soil physiochemical properties affecting plant growth promotion (PGP) by P. radicum were identified. This knowledge is already being used by growers to target application of P. radicum to soil types conducive to plant growth responses. Understanding these factors enables a more efficient use of resources ($) by ensuring growers avoid ploughing money into non-responsive soils. Targeting use to responsive soils means inoculant reliability is increased, adding value to the product for Bio-Care Technology and increasing grower adoption.

2. Advances in understanding of the core mechanisms of PGP by P. radicum were made. Previous research proposed that P solubilisation by the fungus was a major mechanism driving PGP. Several lines of experimental enquiry have shown that this hypothesis is tenuous. Accordingly, P. radicum should not be used as an inoculant to increase efficiency of P-fertilisers, nor to increase plant growth via soil P release. The mechanism appears to be a general PGP (phytohormone secretion - S. Anstis, Adelaide Uni/CSIRO).

The inoculant may be used across a range of different soils; there is no requirement for a high level of unavailable P. As such, the potential ‘market’ for P. radicum is greater than originally thought. Growers are advised against using P. radicum to manage P inputs, thereby saving yield loss costs associated with under-fertilising crops.

3. New strains of Penicillium have been discovered that solubilise high rates of P from inorganic minerals and increase growth of different crop and pasture species.

The newly discovered fungi appear to have potential to increase the yield and P nutrition of Australian crops (what P. radicum was initially proposed to do). Use of these fungi may lead to increased P-fertiliser use efficiency (cost savings) and release of unavailable P from soils (recovering P from previous fertiliser applications).

4. P availability is a key factor limiting the productivity (and therefore profitability) of organic farming across Australia. Organic growers cannot use soluble inorganic P fertilisers but must rely on less readily-available forms of the nutrient (e.g. rock phosphates).

The newly discovered Penicillium fungi may be used by organic growers to increase P release from rock P. Given the specific problem these growers have maintaining P nutrition, these new biologicals have much potential to increase the viability of broad-acre organic farming in Australia.

**Environmental outcomes**
P fertilisers are a non-renewable resource. Gaining higher plant-use efficiency from the resource is essential for long-term agricultural sustainability. As more 'available' sources of P fertilisers are exhausted, other less-available forms of the nutrient must be explored, increasing the cost of fertilisation.

By accessing the P that has accumulated from historical applications, risks associated with the movement of P from soils into natural ecosystems may be reduced.

Achievements/Benefits

Background

The availability of phosphorus to plants is a key constraint to agricultural production across much of Australia. Depending on particular geochemical attributes, P in soils may be immobilised by binding with cations (Ca, Mg, Fe, Al, etc.), adsorption to metal oxides, hydroxides or silicates or via precipitation reactions. Consequently, the efficiency of P-based fertilisers is very low, with only 10-30% of the P applied in fertiliser captured by plants in the year of application. High application rates of P fertiliser are therefore required to sustain adequate P nutrition to plants. In Australia approximately $600 million is spent on P-based fertilisers annually and an estimated $10 billion worth of P has gradually accumulated in agricultural soils.

The inefficiency of phosphate fertilisers is of obvious economic importance, affecting farm profitability and ongoing land management decisions. However, environmental concerns are even more important, particularly as agricultural production is increasingly expected to comply with accredited environmental management systems. One such concern is the movement of ecologically-significant loads of P into waterways: typically via movement of soil high in P during erosion or run-off events. Such issues are fundamental to the sustainable management of Australian agricultural systems. Therefore, environmentally benign approaches to increasing the efficiency of P fertilisers and releasing P bound in soil for plant use are highly desirable and represent the underlying objective of this research project.

Soil microorganisms are fundamentally involved in the processes of transforming immobilised soil P to plant-available forms. Research is underway to determine the potential for exploitation of particular microorganisms to increase plant growth on soils high in immobilised P. Much of this work has focused on fungi in the genus *Penicillium*, as many species of *Penicillium* have been found to strongly solubilise inorganic phosphates. Researchers at Agriculture and Agrifood Canada (AAFC) discovered and developed a strain of *P. bilaiae* with strong phosphate solubilising activity. This fungus has now been commercially developed into a seed inoculant by PhilomBios Inc (Saskatoon) to promote better use of soil phosphate. Growers using *P. bilaiae* as a seed coating (in a manner similar to rhizobium) achieve higher crop yields (7.6% increase in more than 400 trials; 95% confidence level) and greater phosphate nutrition of the crop plants.

The success of *P. bilaiae* in North America stimulated similar work worldwide. In Australia a new species of fungus was discovered on wheat roots and was named *P. radicum* (Charles Sturt University and Australian Seed Inoculants Ltd). *P. radicum* has the capacity (in vitro) to solubilise a wide range of inorganic phosphates commonly found in soil, though the rate of P-solubilisation by *P. radicum* is probably too low to actually affect plant growth. The prime mechanisms for this solubilisation are centred on the secretion of gluconic acid, which can mobilise P from insoluble sources via pH reduction or chelation-based mechanisms. Successful demonstration of plant growth promotion in glasshouse and field trials culminated in the development of *P. radicum* as the inoculant Pr70 Release (Bio-Care Technology Pty Ltd, Somersby, NSW). In 2002, sufficient inoculum was produced to treat 3,000 tonnes of wheat seed. This production level was high enough to enable extensive testing by growers, so that the best opportunities for widespread use (soil type, agro-ecological conditions, etc) in Australia could be identified.

This investigation (Project CSO223) was based on an investigation of the soil microbial ecology of *P. radicum*. This involved determining the effects of various soil properties on plant growth promotion by *P. radicum* and relating this to the growth responses observed in field and glasshouse trials. Knowledge generated in the project can be directly transferred to growers and other land managers to allow informed decision making as to when and where *P. radicum* should be used. Ultimately, this was envisioned to provide higher efficiency of P fertilisers, improve crop yields (especially wheat) on P-retaining soils and reduce the accumulation of P in agricultural soils with a concomitant reduction in related environmental impacts.

There were two main research themes (outcomes) to the current project:
1. Investigating the ecology of *P. radicum*, an Australian plant growth-promoting fungal inoculant
2. Isolation and characterisation of new fungi associated with wheat roots with the aim of identifying isolates with high P solubilising activity that may be used in preference to *P. radicum* (higher P-solubilisation or plant growth promotion ability)
or in conditions not suitable for *P. radicum* (e.g. different soil types).

**Key findings/outcomes**

In *in vitro* assays, the rate of P-solubilisation by *P. radicum* was low compared to newly isolated *Penicillium* fungi. Despite this, *P. radicum* was able to promote strong plant growth of wheat in specific soil conditions. Analysis of wheat shoot tissue often showed no differences in P uptake between inoculated and non-inoculated plants, indicating the link between soil P solubilisation and PGP by *P. radicum* is weak. These data are supported by previously published research in which, under field conditions, the PGP of *P. radicum* could not be saturated with P fertiliser addition; i.e., even under conditions of high P addition, *P. radicum* inoculation still increased plant growth and yield. Accordingly, there must be other mechanisms by which *P. radicum* stimulates plant growth.

S. Anstis (CSIRO/Adelaide Uni), recently provided evidence for phytohormone production by *P. radicum*. It is known that production of such compounds by microorganisms can strongly stimulate root branching, providing a larger surface area through which the plant may access nutrients already available in the soil solution. Therefore, although *P. radicum* inoculation may enable plants to capture more nutrients (including P) from the soil it probably does not increase the plant availability of these nutrients per se. Accordingly, although *P. radicum* can be used to stimulate plant growth and yields, it should not be used as a P-solubilising inoculant and should not be used by growers to manage fertiliser inputs.

The major physical, chemical and biological factors controlling plant growth promotion by *P. radicum* were investigated in a range of experiments conducted *in vitro* and in controlled environment chambers. The results from these experiments can be used as a guide for accurate decision making by growers as to when and where they should use the inoculant. Together with a greater understanding of the key mechanisms of plant growth promotion (PGP), this knowledge can be applied to increase the reliability of *P. radicum* inoculant for growers and the value of the inoculant to the manufacturers.

Marker genes have been inserted into the genome of *P. radicum* using molecular technology. These markers enable unambiguous detection of *P. radicum* in samples from the environment. In addition, a DNA ‘fingerprint’ of *P. radicum*, based on an rDNAsequence, was developed. Using this fingerprint, a second strain of *P. radicum* was discovered. These marker and ‘fingerprint’ technologies can be used by Bio-Care Technology to protect their rights to the exclusive use of the fungus as an inoculant.

An extensive survey of *Penicillium* fungi associated with the roots of wheat plants grown in Australia was undertaken. Laboratory screening of isolates (>800) identified a selection of elite mineral-solubilising isolates of *Penicillium*. Many of these isolates were several-fold more effective than *P. radicum* at releasing P from insoluble minerals *in vitro*. The very best of the isolates screened was identified as a strain of *P. bilaiae*, the same species that has been successfully developed as an inoculant in Canada. This is a significant discovery. *P. bilaiae* is established as a commercial success overseas (demonstrated efficacy, economic production, etc.) and an Australian-adapted strain may be similarly used to boost crop production and increase fertiliser efficiency. Furthermore, this screening process resulted in the discovery of a previously unidentified species of *Penicillium* with strong plant growth promoting activity. After extensive glasshouse screening, these new strains of *Penicillium* were shown to increase the growth of several different agricultural crop plants including a grain cereal (wheat), pulse legume (lentil) and a pasture legume (medic). All of these plant growth responses occurred in soil types where *P. radicum* is ineffective. In the first round of field trials, carried out on a dark alkaline soil in Western Australia, inoculation of lentil with *P. bilaiae* increased crop yield by approximately 10% (2003).

Overseas, the development of phosphate-related inoculants has represented a major step towards increasing crop production on phosphate-retaining soils and increasing the overall efficiency of phosphate fertilisers. This project has greatly contributed to the development of such biological tools in Australia to support the environmentally sustainable management of our soil resources.

**Other research**

Research and development opportunities that emerged during the course of the project were:

(I) Intensively investigate the new *Penicillium* isolates for plant growth in glasshouse trials and in the field. This is already being partially addressed though the GRDC Soil biology Initiative inoculants testing program. However, these trials shed no light on how yield increases are achieved (mechanism of action) nor explore interactions between the plant, microbe and the
environment (ecology, limits of performance, etc.). The possibility of sub-licensing this strain to PhilomBios for use in Australia may also be investigated.

(2) We have shown that the new strains of *Penicillium* are very effective at solubilising phosphorus and can increase plant growth and P uptake and it would be desirable to quantify the release of P in soil following inoculation with the fungal isolates. It would also be desirable to quantify the amount of extra P that can be captured by an inoculated plant following a fertiliser application following inoculation (fertiliser efficiency gains).

(3) Expansion onto crops other than wheat. The newly discovered isolates of *Penicillium* increase the growth of wheat, medic and lentil. Other crops are yet to be tried, but it seems likely the isolates can increase the growth of a range of agriculturally important plant species. Can these inoculants be used to increase productivity and profitability across all phases of the cropping system?

(4) There is scientific evidence of synergistic interactions between *Penicillium* inoculants and *Rhizobia* on legumes. In Canada, the addition of *Penicillium* to *Rhizobia*-based inoculants is commonplace, and is seen as ‘adding value’ to *Rhizobia* products. Such interactions should be investigated to see if the newly discovered *Penicillium* strains can add value to Australia rhizobium-based products. *Rhizobia* products with added *Penicillium* may be a convenient vehicle with which to introduce *Penicillium* technology to the marketplace.

(5) Description of *Penicillium* sp. KC6W2. Fungal strain KC6W2 cannot presently be ascribed to any known species of *Penicillium*. As such, there is potential to patent the fungus as a new species. This would require description of the fungus as a new species, necessitating a detailed study of its biology and taxonomy.

(6) Description of *P. bilaiae* RS7BSD1. This strain of *Penicillium* has strong P solubilisation and PGP abilities. Currently the use of all *P. bilaiae* strains as phosphate-solubilising inoculants is protected by an international patent held on the Canadian isolate (strain PB50 - held by PhilomBios). If it is possible to determine that RS7BSD1 is unique, perhaps an Australian subspecies or with different mechanisms of action to the Canadian isolate, then it may be possible to use this strain in Australia. Alternatively, the possibility of sub-licensing *P. bilaiae* RS7BSD1 to PhilomBios for use in Australia could be investigated.

**Intellectual property summary**

The GRDC and Bio-Care Technology have been asked for ‘clearance’ prior to the publication of any new information arising from this project. A document outlining the IP generated in the project and its implications was delivered to Bio-Care Technology and the GRDC in July 2003.

New species of *Penicillium* fungus were identified with strong P-solubilisation and PGP characteristics. Identification of these fungi has been kept deliberately vague so as not to jeopardise patentability. These isolates are being held by CSIRO and Bio-Care Technology under a biological Material Transfer Agreement (MTA).

The GRDC is investigating opportunities for commercial development of the new *Penicillium* strains.

**Additional information**

List of communications associated with this project CSO223.

Peer reviewed scientific papers:


Peer reviewed conference papers/proceedings: